

specimens of *S. marinus* were below those of non-fertilized specimens and suggested that the difference was related either to incomplete fertilization or to presence of nonviable eggs which are subsequently resorbed after fertilization. MacGregor (1970) observed undeveloped or unfertilized oocytes from the same batch as developing embryos in all species of *Sebastes* examined, but these accounted for only 0.06% of the egg count in *S. paucispinis*. In *S. entomelas*, this percentage was higher (Table 2). Moreover, since the percentage of expected fecundity decreases with later developmental stage, resorption of nonviable embryos may occur throughout the gestation period. Because estimated and realized fecundity may differ, Gunderson (1977) suggested that fecundity estimates of *S. alutus* be considered tentative. Foucher and Beamish (1980) have made similar suggestions concerning fecundity of the oviparous Pacific hake, noting that nonviable oocytes could contribute to overestimates of fecundity. In the genus *Sebastes* it would thus be interesting to determine fecundity in various stages of developing and fertilized ovaries in a shallow living species which could be captured with no fear of extrusion-related reductions in counts of fertilized eggs or embryos.

Acknowledgments

This work was supported by Contract Number 81-ABC-00144 from the National Marine Fisheries Service, Northwest and Alaska Fisheries Center, Seattle, Wash. We thank the fishing industry in Newport, Oreg., for cooperation with sampling and catch information and R. L. Demory and W. H. Lenarz for their helpful comments on the manuscript. We also thank W. H. Lenarz for supplying ages for the specimens from this study and Karen Dykes for typing the drafts of the manuscript.

Literature Cited

- ALVERSON, D. L., A. T. PRUTER, AND L. L. RONHOLT.
1964. A study of demersal fishes and fisheries of the northeastern Pacific Ocean. H. R. MacMillan Lecture Series on Fisheries. Inst. Fish. Univ. B.C., Vancouver, 190 p.
- BAGENAL, T. B., AND E. BRAUM.
1968. Eggs and early life history. In W. E. Ricker (editor), *Methods for assessment of fish production in fresh waters*, p. 159-181. Int. Biol. Programme Handb. 3.
- FOUCHER, R. P., AND R. J. BEAMISH.
1980. Production of nonviable oocytes by Pacific hake (*Merluccius productus*). *Can. J. Fish. Aquat. Sci.* 37: 41-48.
- GUNDERSON, D. R.
1977. Population biology of Pacific ocean perch, *Sebastes alutus*, stocks in the Washington-Queen Charlotte Sound region, and their response to fishing. *Fish. Bull., U.S.* 75:369-403.
- GUNDERSON, D. R., P. CALLAHAN, AND B. GOINEY.
1980. Maturation and fecundity of four species of *Sebastes*. *Mar. Fish. Rev.* 42(3-4):74-79.
- GUNDERSON, D. R., AND T. M. SAMPLE.
1980. Distribution and abundance of rockfish off Washington, Oregon, and California during 1977. *Mar. Fish. Rev.* 42(3-4):2-16.
- MACGREGOR, J. S.
1970. Fecundity, multiple spawning, and description of the gonads in *Sebastes*. U.S. Fish Wildl. Serv., Spec. Sci. Rep. Fish. 596, 12 p.
- PHILLIPS, J. B.
1964. Life history studies on ten species of rockfish (genus *Sebastes*). Calif. Dep. Fish Game, Fish Bull. 126, 70 p.
- RAITT, D. F. S., AND W. B. HALL.
1967. On the fecundity of the redfish, *Sebastes marinus* (L.). *J. Cons.* 31:237-245.

GEORGE W. BOEHLERT

Oregon State University
Marine Science Center
Newport, OR 97365

W. H. BARSS

Oregon Department of Fish and Wildlife
Newport, OR 97365

P. B. LAMBERSON

Oregon State University
Marine Science Center
Newport, OR 97365

A COMPARATIVE STUDY OF AUTOCHTHONOUS BACTERIAL FLORA ON THE GILLS OF THE BLUE CRAB, *CALLINECTES* *SAPIDUS*, AND ITS ENVIRONMENT¹

The bacterial flora of blue crabs, *Callinectes sapidus*, has been previously enumerated and identified by examining blue crab hemolymph (Tubiash et al. 1975; Sizemore et al. 1975; Colwell et al. 1975). Other studies on live blue crabs

¹Contribution No. 82-17C of the Southeast Fisheries Center Charleston Laboratory, National Marine Fisheries Service, NOAA, P.O. Box 12607, Charleston, SC 29412-0607.

have been concerned with the presence of specific human or fishery pathogens in the hemolymph, necrotic tissue, or gill material (Rosen 1967; Williams-Walls 1968; Krantz et al. 1969; Cook and Lofton 1973; Johnson 1976). The statement of Tubiash et al. (1975) that the hemolymph of most healthy blue crabs contains a natural or autochthonous bacterial flora has been challenged, and it has been suggested that further experiments using minimally stressed crabs would be needed to substantiate that statement (Johnson 1976).

This study was designed to determine, seasonally, the natural *Vibrio*, fecal coliform, and aerobic, heterotrophic bacterial populations on blue crabs from environments that differed in salinity and influx of urban and industrial pollutants. These microbial populations were also compared with those found in intertidal oysters (*Crassostrea virginica*), waters, and sediments collected simultaneously with the crabs. Blue crab gills were chosen as a suitable substrate for microbiological investigations because they have direct contact with the environment and are easily sampled and processed for enumeration of their bacterial flora.

Materials and Methods

Two South Carolina areas, Charleston harbor and St. Helena Sound, an estuary 35 mi south of Charleston, were surveyed during a 22-mo period between August 1979 and May 1981 (Fig. 1). Within each area, two sampling stations were selected, representing mean salinities of 10‰ and 25‰. The Charleston harbor sampling stations were Foster Creek, salinity 10‰, a tributary to the Wando River, which, along with the Ashley and Cooper Rivers, forms Charleston harbor; and Shutes Folly Island, salinity 25‰, situated near the center of Charleston harbor. The oyster beds in both sites are closed to harvesting because fecal coliform levels, monitored in the water column and oyster meats by the South Carolina Department of Health and Environmental Control, exceed safe limits for harvesting areas. Foster Creek receives negligible industrial pollution, whereas the Shutes Folly Island station receives a moderate-to-heavy influx of industrial pollutants. The St. Helena Sound stations were the Ashepoo River at Mosquito Creek, salinity 10‰, and St. Helena Sound at the mouth of Rock Creek, salinity 25‰. Shellfish beds at these stations are open to harvesting,

with no discernible influx of urban, industrial, or commercial pollutants. Each station was sampled on a quarterly basis to coincide with the highest and lowest temperatures in the water column and during the middle of the two transitional periods in water temperatures.

Blue crabs were captured in commercial-type crab pots baited with fresh fish heads. The crab pots were harvested from 4 to 24 h after being set, dependent on the seasonal rate of blue crab capture. Intertidal oysters and sediments from the oyster beds were collected manually at low tide. Two hundred grams of the top centimeter of sediment were removed with sterile tongue depressors and placed in sterile containers. Surface water samples (1 m below the surface) were collected with a Niskin² sterile bag sampler (General Oceanics, Miami, Fla.) at the site of blue crab collections. All sediment, water, and oyster samples were immediately cooled with ice. Blue crabs were maintained at their in situ temperature by placing them in a thermally insulated container. All samples were analyzed within 4 to 8 h. During a survey, two to four representative composite samples of blue crab gills (dependent on seasonal activity of blue crabs), three oyster composites, and two samples each of sediment and surface water were collected and analyzed for each sampling station. A composite gill sample contained gills from 10 to 12 crabs. Since mature female blue crabs migrate to higher salinities, male blue crabs dominated the population at 10‰ salinity and females at 25‰ salinity. Whenever possible, blue crabs above the legal harvesting size for South Carolina, 127 mm, were sampled. Each survey was completed within 4 d. For monitoring purposes, temperature and salinity of the surface water were measured, using a YSI Model 33 Salinity-Conductivity-Temperature meter (Yellow Springs Instrument Co., Yellow Springs, Ohio).

Preparation of oyster and water samples for analyses followed standard procedures (American Public Health Association 1970, 1976). Carapaces of the blue crabs were cracked vertically with a blow from a stainless steel knife. The knife did not penetrate into the gut or organs. The carapace was then removed by pulling up on the lateral spines (Fig. 2). Exposed gills were aseptically removed with forceps and placed in

²Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

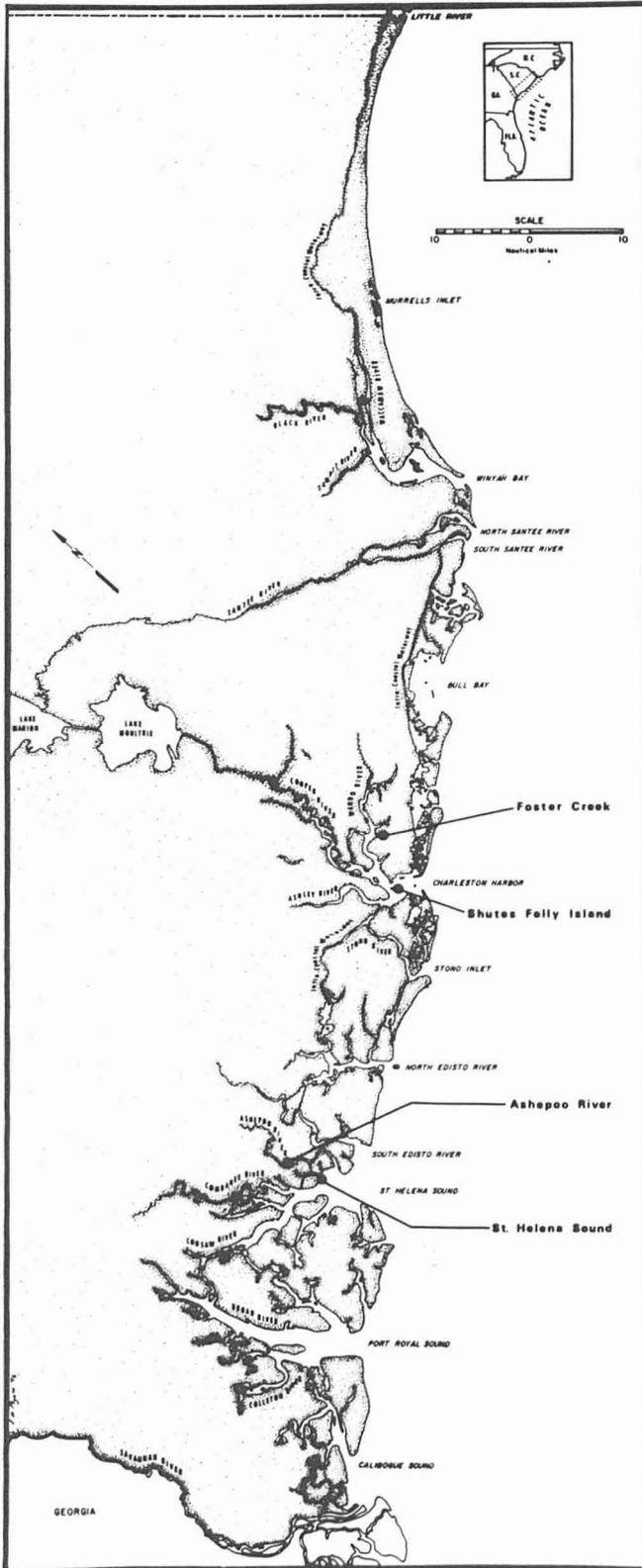


FIGURE 1.—Map of coastal South Carolina showing location of sampling stations.

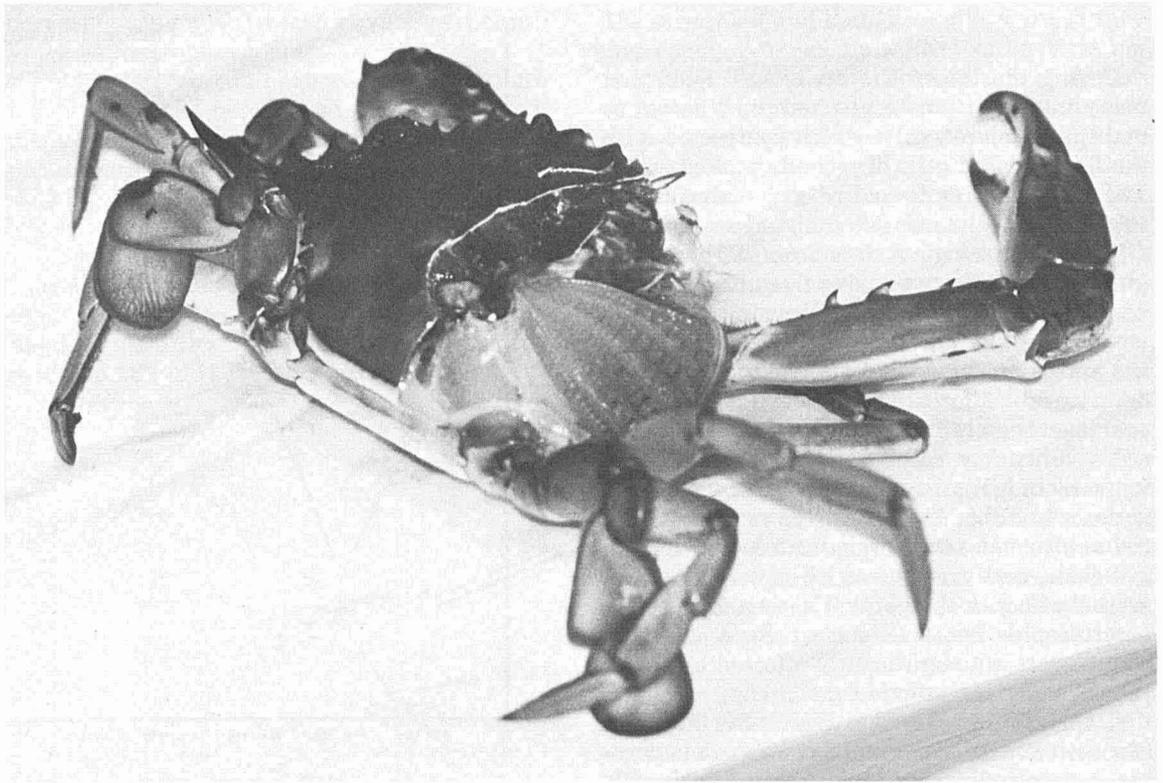


FIGURE 2.—Photograph of blue crab with section removed from vertically cracked carapace.

sterile containers. The blue crab gills were homogenized in a blender for 2 min at high speed and further prepared for bacterial analysis following the standard procedure for oysters. Blue crab gills and oysters were analyzed on a wet-weight basis (50 g), but sediments were analyzed on a volume-to-volume basis (50 ml) because of the great differences in sediment densities found in the environment. The initial dilution was made by volume displacement of the diluent by the sediment in a calibrated container as previously described (Babinchak et al. 1977). All dilutions were made using sterile 0.1% peptone (Difco Laboratories, Detroit, Mich.) saline solution (1.5% NaCl).

Total viable, aerobic, heterotrophic bacterial counts for all samples were determined, using the spread-plate technique and a modification of a low-nutrient, artificial seawater plating medium (ASWLN) of Litchfield et al. (1975) containing the following ingredients per liter of half-strength artificial seawater (Rila Marine Mix, Teaneck, N.J.): 0.5 g peptone (Difco), 0.5 g yeast extract (BBL Microbiology Systems,

Cockeysville, Md.), 0.1 g sodium glycerophosphate (MCB Manufacturing Chemist, Inc., Cincinnati, Ohio), and 20 g agar (BBL). Three replicates of each dilution were plated, and the inoculated plates incubated at 20°C for 14 d.

Fecal coliform counts in all samples were estimated by the three-tube most-probable-number (MPN) procedure prescribed for seawater and tissues (American Public Health Association 1970, 1976). Lauryl sulfate tryptose broth (BBL) was used in the presumptive test, with confirmation in EC broth (BBL) incubated at 44.5°C in a circulating water bath.

Vibrio-like organisms were enumerated on thiosulfate citrate bile salts agar (TCBS; BBL) using the spread-plate technique for all samples. Fifteen to thirty colonies, representing all colonial types, as determined with oblique or darkfield illumination through a stereomicroscope, were picked only from the blue crab gill-inoculated TCBS plates. The cultures were purified and then characterized biochemically using the API 20E system (Analytab Products, Inc., Plainview, N.Y.).

Results

During the initial survey, it was noted that many intermolt blue crabs had dark brown or mahogany-colored gills which contrasted with the light-colored gills of recently molted crabs. The light- and dark-colored gill material was subsequently divided and analyzed separately.

The microbiological data from 61 blue crab gill samples collected during five quarterly surveys were analyzed statistically using a generalized analysis of variance (ANOVA) employing the maximum likelihood approach. The dependent variables for analysis were the microbial counts on the blue crab gills; independent variables were urban and industrial pollution, salinity, season, gill color, and their pairwise interactions. In Table 1, the *P*-values resulting from the analysis of variance indicated that season, gill color, and the interaction of pollution and season affected the total *Vibrio* and aerobic, heterotrophic bacterial counts. Fecal coliform counts were not significantly affected by any of the variables investigated.

Graphically displayed in Figure 3 is the effect of season and gill color on total *Vibrio* and aerobic, heterotrophic bacterial counts. Dark gills had consistently higher counts, and the counts showed a similar pattern with season.

Surprisingly, the absence of urban fecal pollution had no significant impact on fecal coliform counts in blue crab gills (Table 1). Blue crab gills obtained from St. Helena Sound, a pristine area, yielded high fecal coliform counts, whereas intertidal oysters and waters sampled concurrently were relatively free of contamination (Table 2). To confirm the identity of the fecal coliforms found in St. Helena Sound, 90 bacteria were isolated from positive EC broth MPN tubes, checked for their Gram reaction, and analyzed

TABLE 1.—*P* values resulting from generalized ANOVA of crab gill data. The hypothesis that a factor has an effect on microbial counts in blue crab gills is accepted when $P \leq 0.05$.

Independent variables	<i>P</i> -values, dependent variables		
	Heterotrophs	<i>Vibrio</i>	Fecal coliforms
Pollution	0.748	0.233	0.304
Salinity	0.549	0.207	0.211
Season	0.030	0.015	0.355
Gill color	0.001	0.001	0.312
Pollution - salinity	0.916	1	1
Pollution - season	0.001	0.036	1
Pollution - gill color	1	1	1
Salinity - season	1	1	1
Salinity - gill color	1	1	1
Season - gill color	1	1	1

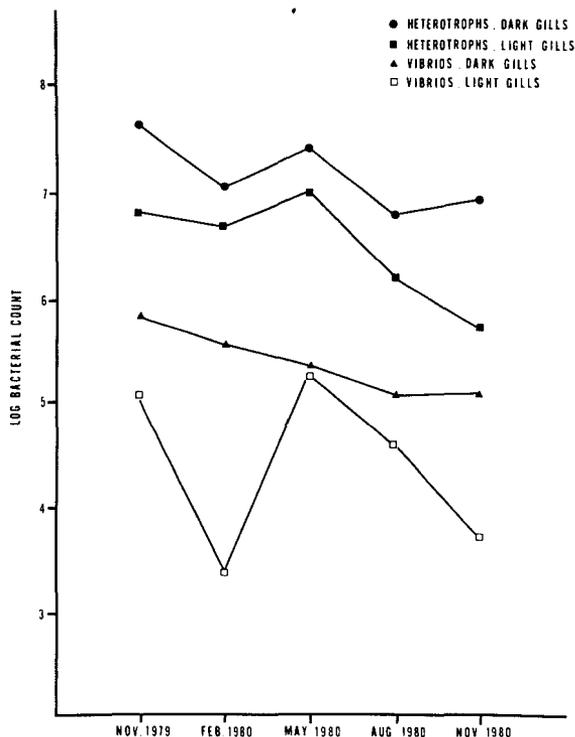


FIGURE 3.—Average total *Vibrio* and heterotrophic bacterial counts per gram of light and dark crab gill tissue from all samples collected from the St. Helena Sound area.

biochemically with the four reactions which constitute the IMViC differential test (American Public Health Association 1976). The sources for the bacterial isolates were blue crab gills (35), water (26), sediment (15), and oysters (14). Ninety-four percent of the isolates were identified as typical *Escherichia coli* and 6% as typical *Enterobacter aerogenes*. Twenty fecal coliforms isolated from blue crab gills were also tested with the API 20E system, and all identified as *E. coli*. Representative samples of blue crab stomach contents analyzed in parallel with the

TABLE 2.—Distribution of fecal coliforms in samples collected from St. Helena Sound area.

Sample	Fecal coliforms/100 g ¹				
	Nov. 1979	Feb. 1980	May 1980	Aug. 1980	Nov. 1980
Intertidal oysters	21	12	12	21	160
Water	80	5	2	16	12
Sediment	430	340	60	19	90
Blue crab gills	2,000	4,300	230	4,300	210

¹100 ml of water and sediment samples.

corresponding gill tissues gave significantly lower heterotrophic counts.

Dark blue crab gills harbored the same heterotrophic bacterial populations found in the sediments (Fig. 4). Similar results with total *Vibrio* populations were obtained in these areas. As shown in Figure 4, oysters and water contained lower heterotrophic counts.

Only 10 to 30% of the TCBS bacterial isolates from individual samples of light and dark gills could be identified to genus and species using the API identification system. *Aeromonas* spp. made up 28% of those TCBS isolates identified.

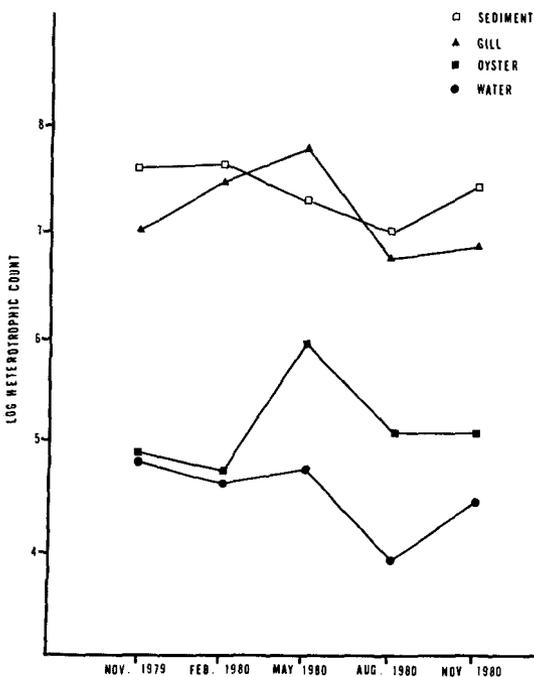


FIGURE 4.—Average heterotrophic bacterial counts per gram of sample collected quarterly from Foster Creek.

Discussion

Common external features which distinguish blue crabs with brown to mahogany gills are rust-spotted exoskeletons and, occasionally, attached barnacles and algae. These conditions are not considered abnormal for late intermolt crabs. Johnson (1977) has also described a viral disease in which blue crabs displayed similar diagnostic signs: failure to molt, a brown-spotted exoskeleton, and gills that were often red-brown.

Sections made from dark gills collected during

the survey showed that these gills were, to varying degrees, fouled by a layer of bacteria and mucus (P. T. Johnson³). Observations of similarly fouled gills of rock crabs, using scanning electron microscopy, showed large numbers of bacteria residing on the gill tissue, similar to those found on blue crab gills in this study using bacterial enumeration procedures (F. Thurberg⁴).

These enumeration data suggest that the gills of blue crabs provide an ecological niche for the growth and physiological activity of heterotrophs, *Vibrio* spp., and related organisms, equivalent to that described for sediments and zooplankton (Kaneko and Colwell 1975 a, b, 1978). The high fecal coliform population found in our pristine area would indicate that gill surfaces also provide a protective ecological niche much like that reported for marine sediments, where high fecal coliform populations can accumulate and persist even in ecosystems where influx of fecal coliforms is low (Rittenburg et al. 1958; Van Donsel and Geldreich 1971; Babinchak et al. 1977).

Urban and industrial pollution did not have an effect on total *Vibrio* and aerobic, heterotrophic bacterial counts on blue crab gills. Since these two microbial populations are indigenous and dependent on nutrient levels for growth, industrial and domestic pollution would not necessarily have shown an effect over the pristine areas sampled. The vast marshlands which drain into the St. Helena Sound area (Tiner 1977) would introduce large natural levels of dissolved and particulate organic material and other nutrients which could support the high bacterial levels observed.

The low rates of identification by the API 20E system can be attributed primarily to the high percentage of clinical bacterial isolates which form the API data base. Even some of our positive identifications are now in question, because marine isolates identified as *Aeromonas* spp. with the API system are known to be Group F vibrios (Seidler et al. 1980). This group of *Vibrio*-like organisms has been associated with diarrheal illness, although the epidemiology of the disease has not been well-defined.

³P. T. Johnson, Northeast Fisheries Center, National Marine Fisheries Service, NOAA, Oxford, MD 21654, pers. commun. 1981.

⁴F. Thurberg, Northeast Fisheries Center, National Marine Fisheries Service, NOAA, Milford, CT 06460, pers. commun. 1981.

The data obtained in this study establish blue crab gills as excellent surfaces for enumerating the blue crab's natural adherent bacterial population. Bacterial quantitation, which is difficult to achieve with other crab surfaces, is easily accomplished with gills. Succession of bacterial species and the possible influence of environmental contaminants in the bacterial colonization of blue crab gills are also conveniently accommodated by the molting process. The freshly molted gill surfaces can be compared with gills that have been exposed to the environment for extended periods. Gill surfaces may provide a model system for monitoring biological or chemical pollutants based on observable changes in the autochthonous bacterial populations of blue crab gills.

Acknowledgments

The authors thank L. Ng for statistical analysis of the data and V. Ward and D. Green for their technical assistance.

Literature Cited

- AMERICAN PUBLIC HEALTH ASSOCIATION.
1970. Recommended procedures for the examination of sea water and shellfish. 4th ed. Am. Public Health Assoc., Inc., Wash., D.C.
1976. Compendium of methods for the microbiological examination of foods. Am. Public Health Assoc., Inc., Wash., D.C.
- BABINCHAK, J. A., J. T. GRAIKOSKI, S. DUDLEY, AND M. F. NITKOWSKI.
1977. Distribution of faecal coliforms in bottom sediments from the New York Bight. Mar. Pollut. Bull. 8: 150-153.
- COLWELL, R. R., T. C. WICKS, AND H. S. TUBIASH.
1975. A comparative study of the bacterial flora of the hemolymph of *Callinectes sapidus*. Mar. Fish. Rev. 37 (5-6):29-33.
- COOK, D. W., AND S. R. LOFTON.
1973. Chitinoclastic bacteria associated with shell disease in *Penaeus* shrimp and the blue crab (*Callinectes sapidus*). J. Wildl. Dis. 9:154-159.
- JOHNSON, P. T.
1976. Bacterial infection in the blue crab, *Callinectes sapidus*: Course of infection and histopathology. J. Invertebr. Pathol. 28:25-36.
1977. A viral disease of the blue crab, *Callinectes sapidus*: Histopathology and differential diagnosis. J. Invertebr. Pathol. 29:201-209.
- KANEKO, T., AND R. R. COLWELL.
1975a. Adsorption of *Vibrio parahaemolyticus* onto chitin and copepods. Appl. Microbiol. 29:269-274.
1975b. Incidence of *Vibrio parahaemolyticus* in Chesapeake Bay. Appl. Microbiol. 30:251-257.
1978. The annual cycle of *Vibrio parahaemolyticus* in Chesapeake Bay. Microb. Ecol. 4:135-155.
- KRANTZ, G. E., R. R. COLWELL, AND E. LOVELACE.
1969. *Vibrio parahaemolyticus* from the blue crab *Callinectes sapidus* in Chesapeake Bay. Science (Wash., D.C.) 164:1286-1287.
- LITCHFIELD, C. D., J. B. RAKE, J. ZINDULIS, R. T. WATANABE, AND D. J. STEIN.
1975. Optimization of procedures for the recovery of heterotrophic bacteria from marine sediments. Microb. Ecol. 1:219-233.
- RITTENBERG, S. C., T. MITTWER, AND D. IVLER.
1958. Coliform bacteria in sediments around three marine sewage outfalls. Limnol. Oceanogr. 3(1):101-108.
- ROSEN, B.
1975. Shell disease of the blue crab, *Callinectes sapidus*. J. Invertebr. Pathol. 9:348-353.
- SEIDLER, R. J., D. A. ALLEN, R. R. COLWELL, S. W. JOSEPH, AND O. P. DAILY.
1980. Biochemical characteristics and virulence of environmental group F bacteria isolated in the United States. Appl. Environ. Microbiol. 40:715-720.
- SIZEMORE, R. K., R. R. COLWELL, H. S. TUBIASH, AND T. E. LOVELACE.
1975. Bacterial flora of the hemolymph of the blue crab, *Callinectes sapidus*: Numerical taxonomy. Appl. Microbiol. 29:393-399.
- TINER, R. W., JR.
1977. An inventory of South Carolina's coastal marshes. S.C. Mar. Resour. Cent. Tech. Rep. 23, 33 p.
- TUBIASH, H. S., R. K. SIZEMORE, AND R. R. COLWELL.
1975. Bacterial flora of the hemolymph of the blue crab, *Callinectes sapidus*: Most probable numbers. Appl. Microbiol. 29:388-392.
- VAN DONSEL, D. J., AND E. E. GELDREICH.
1971. Relationships of salmonellae to fecal coliforms in bottom sediments. Water Res. 5:1079-1087.
- WILLIAMS-WALLS, N. J.
1968. *Clostridium botulinum* type F: Isolation from crabs. Science (Wash., D.C.) 162:375-376.

JOHN A. BABINCHAK
DANIEL GOLDMINTZ
GARY P. RICHARDS

*Southeast Fisheries Center Charleston Laboratory,
National Marine Fisheries Service, NOAA
P.O. Box 12607, Charleston, SC 29412-0607*